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Microwave Radiation (2450-MHz) Potentiates the Lethal Effect of Endotoxin in Mice

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16. ABSTRACT

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MICROWAVE RADIATION (2450-MHz) POTENTIATES THE LETHAL EFFECT OF ENDOTOXIN IN MICE

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Abstract—Groups of male CBA/J mice were injected with *Salmonella typhimurium* lipopolysaccharide (LPS) and irradiated with 2450 MHz (CW) microwaves. The 50% lethal dose (LD_{50}) of LPS was determined for mice irradiated at 30, 20, 10 and 5 mW/cm^2 immediately following injection. The average specific absorption rate was approximately 0.6 W/kg per 1 mW/cm^2 incident power. An equal number of animals served as sham-irradiated controls for each power density. The mice were placed individually in small containers and were maintained at 22°C and 50% relative humidity during a 2 hour irradiation period. Following irradiation the mice were returned to their home cages and were observed for 48 hr. A significant decrease in the LPS dose required to kill 50% of the mice was observed at power densities of 20 and 30 mW/cm^2. High ambient temperature (37°C) also potentiated the lethal effect of endotoxin. Microwave irradiation prior to LPS injection, however, did not affect the lethal action of LPS.

INTRODUCTION

OVER THE past several years significant numbers of non-ionizing radiation (NIR) devices have been marketed which are in use by the general public. One of the most popular devices in the United States is the microwave oven which employs a frequency of 2450 MHz. With the increasing use of such microwave generating devices concern for the public's safety has increased.

It has been demonstrated that high intensity NIR induces thermal loads in laboratory animals which affect the hematologic and immunologic systems (Sm79a). For example, altered blood counts (De64), decreased inflammatory responses (Li77), and changes in the distribution of lymphocyte populations in lymphoid organs (Li79; 80) have been reported following microwave irradiation at intensities which caused significant increases in body temperature. Other studies show that long term exposure of rabbits or mice to low intensity microwave radiation or repeated sessions of whole body microwave hyperthemia in mice result in the lowering of natural antibacterial resistance, suppression of immune responsiveness and increased mortality from experimental acute staphylococcal infections (Sz75; 80). From these latter studies it appears that microwave radiation affects the course of bacterial infections. To further examine this hypothesis, we studied the effects of microwave radiation on the lethality of bacterial endotoxin in mice.

MATERIALS AND METHODS

Animals

Experimentally naive male CBA/J mice between 8 and 12 weeks of age (25–35 gms) were used (Jackson Laboratories, Bar Harbor, Maine). Prior to use, the mice were held in the vivarium for 1 week following shipment.The animals were allowed food and water *ad libitum* and were maintained on a 12:12 light–dark cycle.

Endotoxin treatment

Mice were injected intraperitoneally with endotoxin (*Salmonella typhimurium* lipopolysaccharide (LPS), Difco Laboratories, Detroit, Michigan) at the desired dose in a volume of 0.2 ml pyrogen-free saline. All test tubes and syringes were guaranteed pyrogen-free by the manufacturer. The LPS was boiled for 1 hr prior to use. Groups of mice were injected with 400, 300, 200, 100, 50, 25, 12.5, or 6.5 μg of LPS. These amounts correspond to LPS doses of approx. 13 mg/kg to 0.2 mg/kg. Unless stated otherwise, mice were returned to their home cage or were placed in small vented containers and exposed to microwave radiation, immediately following injection. Control mice were injected with an equal volume of pyrogen-free saline.

Microwave irradiation

Mice were placed individually into small containers, 6 cm on a side, consisting of 1.5 mm thick clear polycarbonate with a polyethylene top. Each side of the container and the top was drilled with 6–8 ventilation holes 5 mm dia. The plastic containers were placed in a 5 $\times$5 array, as described (Ki77, Berm78) on a foamed polystyrene form to ensure their exact positioning in the microwave field (Fig. 1). The animals were exposed at incident power densities of 5, 10, 20 or 30 mW/cm^2 for 120 min in a 2450 MHz CW microwave exposure facility (Sm79b) under controlled environmental conditions (22 $\pm$ 0.5°C, 50 $\pm$ 10% relative humidity and average lineal air velocity of 0.31 m/s). The incident power density was determined using an *E*-field dipole probe (El 75) placed at the center of the array without the presence of animals or containers in the chamber. The average specific absorption rate (SAR) for mice used in this study is comparable to that reported earlier (Ki77, Berm78), in which an identical array and animals of similar mass were used to determine SAR by twin-well calorimetry. The estimated average SAR value for mice in this experiment based on these earlier reports is approx. 0.6 mW/g per 1 mW/cm^2 incident power density.

An equal number of sham-irradiated mice were injected with LPS or saline and placed in a controlled environment chamber adjacent to the microwave irradiation chamber. The irradiated and sham-irradiated animals were handled in a similar fashion throughout the experiments and were housed five per cage with access to food and water prior to and after irradiation. The animals were observed periodically for 48 hr following treatment.

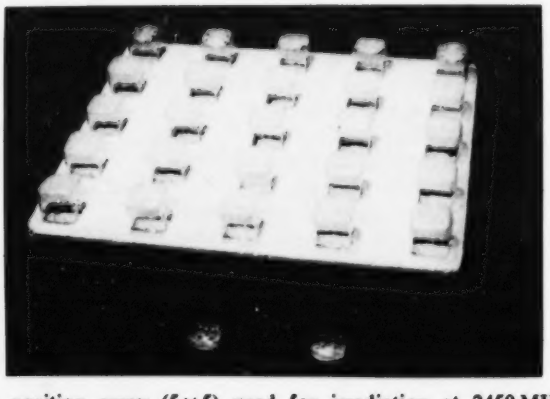

Fig. 1. Mouse position array (5 $\times$ 5) used for irradiation at 2450 MHz. Two vented containers, with covers removed, are shown in the foreground.

Data analysis

Determination of the 50% lethal dose (LD_{50}) of LPS was done using probit analysis and differences between matched irradiated and sham-irradiated mice were determined using the relative potency test (Fi75).

RESULTS

Table 1 shows the results of microwave irradiation on LPS lethality in CBA/J mice. Five groups of 5 mice per group were injected with a single concentration of LPS. Immediately following LPS injection the mice where microwave irradiated. Simultaneously an equal number of LPS-injected mice were sham-irradiated. A total of 10 mice per LPS-concentration was used for each power density except that 20 mice were used for LPS doses of 50 and 100 μg at 30 mW/cm^2.

The data show (Table 1) that as the microwave power density increases above 10 mW/cm^2 the dose of LPS that is required to kill 50% of the animals decreases. This decrease in LPS dose is statistically significant ($P < 0.05$) at 20 and 30 mW/cm^2 compared with their respective sham-irradiated groups. A group of 25 mice that were injected with saline and immediately irradiated at 30 mW/cm^2 for 2 hr all survived this treatment.

Table 2 shows the results of experiments in which mice were injected with LPS within 10 min following microwave irradiation. Under these conditions, there was no apparent change in lethality at 30 mW/cm^2.

The effect that high (37°C) ambient temperature has on LPS lethality is presented in Table 3. Exposure of LPS injected mice to 37°C caused a lowering of the LD_{50} to 152 μg. This is in comparison to a mean LD_{50} of 257 μg for sham-irradiated mice held at 22°C, and to a LD_{50} of 367 μg for home cage controls.

DISCUSSION

This study demonstrates that there was a shift to decreasing concentrations of LPS required to kill 50% of mice irradiated at 2450 MHz microwaves at incident power densities above 10 mW/cm^2. This shift of the LD_{50} from 237 μg LPS at 5 mW/cm^2 to 37 μg LPS at 30 mW/cm^2 is a dramatic decrease. The response of endotoxin treated mice to microwaves appears to have a threshold above 10 mW/cm^2. A significant decrease in LD_{50} dose between sham-irradiated and irradiated mice was seen at 20 and 30 mW/cm^2. This change in LPS LD_{50} was

Table 1. The effect of 2450-MHz microwave irradiation on lipopolysaccharide (LPS) lethality in mice. Mice irradiated with microwaves immediately following LPS injection

Power Density mW/cm^2	LD_{50} (μg LPS) Irradiated	Sham-Irradiated
5	237 (194-278)[a]	257[b]
10	250 (205-293)	292 (232-375)
20	128 (85-177)	248 (194-295)[c]
30	37 (17-60)	230 (183-274)[c]

[a] 95% confidence limit.

[b] 95% confidence limit not estimable by probit analysis because G is by Finney's notation too big (Finney, 1971).

[c] Significant difference in LD_{50} between sham-irradiated and irradiated mice.

Table 2. The effect of 2450-MHz microwave irradiation on lipopolysaccharide (LPS) lethality in mice. Mice irradiated with microwaves immediately prior to LPS injection

Power Density (mW/cm^2)	Lethality[a]			
	400 μg LPS	200 μg LPS	100 μg LPS	50 μg LPS
0	6/6	0/6	0/6	0/6
5	4/6	0/6	0/6	0/6
30	6/6	0/6	0/6	0/6

[a]Number of dead mice divided by number in group.

Table 3. The effect of housing and ambient temperature on lipopolysaccharide lethality in mice

Holding Conditions	LD_{50} (μg LPS)
Home cage control[a]	367 (274-482)[c]
High ambient temperature[b]	152 (44-268)

[a]Mice returned to home cage (6 per cage) immediately following LPS injection.

[b]Mice placed in individual containers and held at 37°C for 2 hours immediately following LPS injection.

[c]95% confidence limit.

seen only when mice were irradiated immediately following LPS injection. Exposure of LPS-injected mice to 37°C caused a lowering of the LD_{50} dose of LPS.

Endotoxins are lipopolysaccharide-protein complexes contained in the cell walls of Gram negative bacteria which are released from the bacteria when the organisms undergo lysis. These toxins are found in both infectious and non-infectious Gram negative bacteria. The presence or absence of the protein component serves to distinguish endotoxins from purified lipopolysaccharides. Endotoxins due to Gram negative bacterial infection are a major medical concern, with an estimated 70,000 fatalities per year in the United States (Mc72).

Endotoxins are capable of producing a myriad of physiological changes in mammals such as fever induction, blood pressure changes, leukocytosis followed by leukopenia and an initial hypercoagulation of blood followed by prolonged clotting time (Mo78). Some endotoxin-induced physiological changes are influenced by environmental factors. For example, it has been shown that heavy metals, (Se66) and changes in environmental temperatures (Con 61; At64; Berr66) increase the sensitivity to bacterial endotoxins.

The intraperitoneal injection of LPS in mice produces a hypothermia within a few minutes (Coo79). In our experiments mice treated with LPS were characterized by crouching, immobility, coolness, ruffled fur, diarrhea and failure to eat or drink. The surviving animals exhibited hypothermia and a characteristic huddled appearance for more than 48 hours following injection.

It has been reported (Con61) that mice given a large dose of endotoxin and subjected to elevated environmental temperatures were more susceptible to the lethal action of endotoxin. Hyperthermia, induced either by physical or pharmacological means, increased the susceptibility of rabbits to the lethal action of endotoxin (At64). Endotoxin may interfere with the thermoregulatory mechanisms by sensitizing mice to cold and heat. It has been reported (Berr66) that injection of endotoxin inhibits the control of body heat loss to a cooler environment due to the effects of endotoxin on metabloism. Furthermore, environmental temperatures below 25°C and above 32°C (the thermal neutral zone of mice) enhanced the lethal action of LPS.

Thermogenic levels of microwave radiation (i.e. power densities that produce measurable core temperature increases) are capable of producing biological changes. It appears that a significant microwave-induced thermal

burden was imposed on the LPS-injected mice irradiated at 20 and 30 mW/cm^2. Apparently LPS predisposed the mice to the thermogenic properties of microwaves. Support for this assumption comes from the experiment in which an ambient temperature of 37°C caused a similar decrease in the LPS LD_{50} dose.

It is interesting to note that when mice were isolated (placed individually in vented containers for 2 hr) immediately following LPS injection, the LPS dose that was required to kill 50% of the animals was lower (Table 1, mean $LD_{50} = 257$ for sham-irradiated mice) than that for mice which were returned to their home cages (Table 3, $LD_{50} = 367$) immediately following LPS injection.

The results demonstrate that mice exposed to high ambient temperature (37°C), thermal loading by microwaves (20 and 30 mW/cm^2) and the stress of isolation are more sensitive to the lethal action of endotoxin. This potentiation of the lethal effects of endotoxin appears to be due to the physiological stress produced by these conditions, however, definitive evidence for this theory requires further work.

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